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(54) Title: HUMAN ANTIBODIES DERIVED FROM IMMUNIZED XENOMICE

(57) Abstract

Fully human antibodies against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenges, but whose endogenous loci have been disabled. Various subsequent manipulations can be performed to obtain either antibodies per se or analogs thereof.

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## HUMAN ANTIBODIES DERIVED FROM IMMUNIZED XENONICE

## Technical Field

5 The invention relates to the field of immunology, and in particular to the production of antibodies. More specifically, it concerns producing such antibodies by a process which includes the step of immunizing a transgenic animal with an antigen to which antibodies are desired. The transgenic animal has been modified so as to produce human, as opposed to endogenous, antibodies.

10 Background Art

PCT application WO 94/02602, published 3 February 1994 and incorporated herein by reference, describes in detail the production of transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than endogenous antibodies in response to antigenic challenge. Briefly, the endogenous loci encoding the heavy and light immunoglobulin chains are inactivated in the transgenic hosts and loci encoding human heavy and light chain proteins are inserted into the genome. In general, the animal which provides all the desired modifications is obtained by cross breeding intermediate animals containing fewer than the full complement of modifications. The preferred embodiment of nonhuman animal described in the specification is a mouse. Thus, mice, specifically, are described which, when administered immunogens, produce antibodies with human variable regions, including fully human antibodies, rather than murine antibodies that are immunospecific for these antigens.

30 The availability of such transgenic animals makes possible new approaches to the production of fully human antibodies. Antibodies with various immunospecificities are desirable for therapeutic and diagnostic use. Those antibodies intended for human therapeutic and *in vivo* diagnostic use, in particular, have been problematic because prior art sources for such antibodies resulted in immunoglobulins bearing the characteristic structures of

antibodies produced by nonhuman hosts. Such antibodies tend to be immunogenic when used in humans.

The availability of the nonhuman, immunogen responsive transgenic animals described in the above-referenced WO 94/02602 make possible convenient production of human antibodies without the necessity of employing human hosts.

# Disclosure of the Invention

10 The invention is directed to methods to produce human antibodies by a process wherein at least one step of the process includes immunizing a transgenic nonhuman animal with the desired antigen. The modified animal fails to produce endogenous antibodies, but instead produces B-cells which secrete fully human immunoglobulins. The antibodies produced can be obtained from the animal directly or from immortalized B-cells derived from the animal. Alternatively, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly or modified to obtain analogs of antibodies such as, for example, single chain F<sub>2</sub> molecules.

Thus, in one aspect, the invention is directed to a method to produce a fully human immunoglobulin to a specific antigen or to produce an analog of said immunoglobulin by a process which comprises immunizing a nonhuman animal with the antigen under conditions that stimulate an immune response. The nonhuman animal is characterized by being substantially incapable of producing endogenous heavy or light immunoglobulin chain, but capable of producing immunoglobulins with both human variable and constant regions. In the resulting immune response, the animal produces B cells which secrete immunoglobulins that are fully human and specific for the antigen. The human immunoglobulin of desired specificity can be directly recovered from the animal, for example, from the serum, or primary B cells can be obtained from the animal and immortalized. The immortalized B cells can be used directly as the source of human antibodies or, alternatively, the genes encoding the

antibodies can be prepared from the immortalized B cells or from primary B cells of the blood or lymphoid tissue (spleen, tonsils, lymph nodes, bone marrow) of the immunized animal and expressed in recombinant hosts, with or without modifications, to produce the immunoglobulin or its analogs.

In addition, the genes encoding the repertoire of immunoglobulins produced by the immunized animal can be used to generate a library of immunoglobulins to permit screening for those variable regions which provide the desired affinity. Clones from the library which have the desired characteristics can then be used as a source of nucleotide sequences encoding the desired variable regions for further manipulation to generate antibodies or analogs with these characteristics using standard recombinant techniques.

In another aspect, the invention relates to an immortalized nonhuman B cell line derived from the above described animal. In still another aspect, the invention is directed to a recombinant host cell which is modified to contain the gene encoding either the human immunoglobulin with the desired specificity, or an analog thereof which exhibits the same specificity.

In still other aspects, the invention is directed to antibodies or antibody analogs prepared by the above-described methods and to recombinant materials for their production.

In still other aspects, the invention is directed to antibodies which are immunospecific with respect to particular antigens set forth herein and to analogs which are similarly immunospecific, as well as to the recombinant materials useful to production of these antibodies.

# Brief Description of the Drawings

Figure 1 is a schematic of the construction of the yHc human heavy chain YAC.

Figure 2 is a schematic of the construction of the yK2 human kappa light chain YAC.

Figure 3 shows the serum titers of anti-IL-6 antibodies from a Xenomouse<sup>®</sup> immunized with human IL-6 and

- which antibodies contain human  $\kappa$  light chains and/or human  $\mu$  heavy chains.
- Figure 4 show the serum titers of anti-TNF $\alpha$  antibodies from a Xenomouse<sup>®</sup> immunized with human TNF- $\alpha$  and which antibodies contain human  $\kappa$  light chains and/or human  $\mu$  heavy chains.
- Figure 5 shows serum titers of anti-CD4 antibodies from a Xenomouse<sup>®</sup> immunized with human CD4 and which antibodies contain human  $\kappa$  light chains and/or human  $\mu$  heavy chains.
- Figure 6 shows the serum titers of a Xenomouse<sup>®</sup> immunized with 300.19 cells expressing L-selected at their surface. In the ELISA assay used, these antibodies are detectable if they carry human  $\mu$  constant region heavy chains.
- Figure 7 shows the serum titers of a Xenomouse<sup>®</sup> immunized with 300.19 cells expressing L-selected at their surface. In the ELISA assay used, these antibodies are detectable only if they carry human  $\kappa$  light chains.
- Figure 8 shows a FACS analysis of human neutrophils incubated with serum from a Xenomouse<sup>®</sup> immunized with human L-selected and labeled with an antibody immunoreactive with human light chain  $\kappa$  region.
- Figure 9 shows a diagram of a plasmid used to transfect mammalian cells to effect the production of the human protein gp39.
- Figure 10 represents the serum titration curve of mice immunized with CHO cells expressing human gp39. The antibodies detected in this ELISA must be immunoreactive with gp39 and contain human heavy chain  $\mu$  constant regions of human  $\kappa$  light chains.
- Figure 11 is a titration curve with respect to monoclonal antibodies secreted by the hybridoma clone D5.1. This clone is obtained from a Xenomouse<sup>®</sup> immunized with tetanus toxin C (TTC) and contains human  $\kappa$  light chain and human  $\mu$  constant region in the heavy chain.

- Figure 12 DNA sequence of the heavy chain of anti-tetanus toxin monoclonal antibody D5.1.4 (a subclone of D5.1). Mutations form germline are boxed.
- Figure 13 DNA sequence of the kappa light chain of anti-tetanus toxin monoclonal antibody D5.1.4. Mutations form germline are boxed.
- Figure 14 shows the serum titers of anti-IL-8 antibodies of "xenomouse" immunized with human IL-8 and which antibodies contain human  $\kappa$  light chains and/or human  $\mu$  heavy chains.
- Figure 15 inhibition of IL-8 binding to human neutrophils by monoclonal anti-human-IL-8 antibodies.
- Figure 16 (A-H) DNA sequences of the heavy chain and kappa light chain of the anti-IL-8 antibodies D1.1 (16A-B), K2.2 (16C-D), K4.2 (16E-F), and K4.3 (16G-H).
- Modes of Carrying Out the Invention
- In general, the methods of the invention include administering an antigen for which human forms of immunospecific reagents are desired to a transgenic nonhuman animal which has been modified genetically so as to be capable of producing human, but not endogenous, antibodies. Typically, the animal has been modified to disable the endogenous heavy and/or kappa light chain loci in its genome, so that these endogenous loci are incapable of the rearrangement required to generate genes encoding immunoglobulins in response to an antigen. In addition, the animal will have been provided, stably, in its genome, at least one human heavy chain locus and at least one human light chain locus so that in response to an administered antigen, the human loci can rearrange to provide genes encoding human variable regions immunospecific for the antigen.
- The details for constructing such an animal useful in the method of the invention are provided in the PCT application WO 94/02602 referenced above. Examples of YACs for the present invention can be found in, for example, Green et al. *Nature Genetics* 7:13-21 (1994). In a preferred

embodiment of the XenonKouse", the human heavy chain YAC, YHIC (1020 kb), and human light chain YAC, YK2 (880 kb) are used. YHIC is comprised of 870 kb of the human variable region, the entire D and J' region, human H, E, and Y2 constant regions and the mouse J' enhancer. YK2 is comprised of 650 kb of the human kappa chain proximal variable region (V<sub>K</sub>), the entire J<sub>K</sub> region, and C<sub>K</sub> with its flanking sequences that contain the kappa deleting element (kde). Both YACs also contain a human HPT selectable marker on their YAC vector arm.

Construction of YHIC and YK2 was accomplished by methods well known in the art. In brief, YAC clones bearing segments of the human immunoglobulin loci were identified by screening a YAC library (Calbertsen et al, EMAS 87:4256 (1990)) overlapping clones were joined by recombination using standard techniques (Mendez et al, GENOMICS 26:294-307 (1995)). Details of the schemes for assembling YHIC and YK2 are shown in Figure 1 and Figure 2 respectively.

YK2 was constructed from the clones A80-C7, A210-F10 and A203-C6 from the Olson library, disclosed in, for example, Burke et al., Science 236:806-812 (1987), Brownstein et al., Science 244:1348-1351 (1989), and Burke et al., Methods in Enzymology 194:251-270 (1991).

For production of the desired antibodies, the first step is administration of the antigen. Techniques for such administration are conventional and involve suitable immunization protocols and formulations which will depend on the nature of the antigen per se. It may be necessary to provide the antigen with a carrier to enhance its immunogenicity and/or to include formulations which contain adjuvants and/or to administer multiple injections and/or to vary the route of the immunization, and the like. Such techniques are standard and optimization of them will depend on the characteristics of the particular antigen for which immunospecific reagents are desired.

As used herein, the term "immunospecific reagents" includes immunoglobulins and their analogs. The term "analog" has a specific meaning in this context. It refers to molecules that contain the fully human portions of the



immunoglobulin which account for its immunospecificity. In particular, complementarily determining regions (CDRs) are required, along with sufficient portions of the framework (FRs) to result in the appropriate three dimensional conformation. Typical immunospecific analogs of antibodies include F(ab')<sub>2</sub>, Fab', and Fab regions. Modified forms of the variable regions to obtain, for example, single chain F<sub>1</sub> analogs with the appropriate immunospecificity are known. A review of such F<sub>1</sub> construction is found, for example, in Huston et al., Methods in Enzymology 203:46-63 (1991). The construction of antibody analogs with multiple immunospecificities is also possible by coupling the variable regions from one antibody to those of second antibody.

The variable regions with fully human characteristics can also be coupled to a variety of additional substances which can provide toxicity, biological functionality, alternative binding specificities and the like. The moieties including the fully human variable regions produced by the methods of the invention include single-chain fusion proteins, molecules coupled by covalent methods other than those involving peptide linkages, and aggregated molecules. Examples of analogs which include variable regions coupled to additional molecules covalently or noncovalently include those in the following nonlimiting illustrative list. Trautnacker, A. et al. Int. J. Cancer Supp (1992) Supp 7:51-52 describe the bispecific reagent Janusin CD4 or to other ligands such as OVCA and IL-7. Similarly, the fully human variable regions produced by the method of the invention can be constructed into F<sub>1</sub> molecules and coupled to alternative ligands such as those illustrated in the cited article. Higgins, P.J. et al. Infect Disease (1992) 166:198-202 described a heteroconjugate antibody composed of OKT3 cross-linked to an antibody directed to a specific sequence in the V3 region of GP120. Such heteroconjugate antibodies can also be constructed using at least the human variable regions contained in the immunoglobulins produced by the invention methods. Additional examples of bispecific

antibodies include those described by Fanger, M.W. et al. *Cancer Treat Res* (1993) 68:181-194 and by Fanger, M.W. et al. *Crit Rev Immunol* (1992) 12:101-124. Conjugates that are immunotoxins including conventional antibodies have been widely described in the art. The toxins may be coupled to the antibodies by conventional coupling techniques or immunotoxins containing protein toxin portions can be produced as fusion proteins. The analogs of the present invention can be used in a corresponding way to obtain such immunotoxins. Illustrative of such immunotoxins are those described by Byers, B.S. et al. *Seminars Cell Biol* (1991) 2:59-70 and by Fanger, M.W. et al. *Immunol Today* (1991) 12:51-54.

It will also be noted that some of the immunoglobulins and analogs of the invention will have agonist activity with respect to antigens for which they are immunospecific in the cases wherein the antigens perform signal transducing functions. Thus, a subset of antibodies or analogs prepared according to the methods of the invention which are immunospecific for, for example, a cell surface receptor, will be capable of eliciting a response from cells bearing this receptor corresponding to that elicited by the native ligand. Furthermore, antibodies or analogs which are immunospecific for substances mimicking transition states of chemical reactions will have catalytic activity. Hence, a subset of the antibodies and analogs of the invention will function as catalytic antibodies.

In short, the genes encoding the immunoglobulins produced by the transgenic animals of the invention can be retrieved and the nucleotide sequences encoding the fully human variable region can be manipulated according to known techniques to provide a variety of analogs such as those described above. In addition, the immunoglobulins themselves containing the human variable regions can be modified using standard coupling techniques to provide conjugates retaining immunospecific regions.

Thus, immunoglobulin "analogs" refers to the moieties which contain those portions of the antibodies of

the invention which retain their human characteristics and their immunospecificity. These will retain sufficient human variable regions to provide the desired specificity. As stated above, all of the methods of the invention include administering the appropriate antigen to the transgenic animal. The recovery or production of the antibodies themselves can be achieved in various ways. First, and most straightforward, the polyclonal antibodies produced by the animal and secreted into the bloodstream can be recovered using known techniques. Purified forms of these antibodies can, of course, be readily prepared by standard purification techniques, preferably including affinity chromatography with protein A, anti-immunoglobulin, or the antigen itself. In any case, in order to monitor the success of immunization, the antibody levels with respect to the antigen in serum will be monitored using standard techniques such as ELISA, RIA and the like. For some applications only the variable regions of the antibodies are required. Treating the polyclonal antiserum with suitable reagents so as to generate Fab', Fab, or F(ab')<sub>2</sub> portions results in compositions retaining fully human characteristics. Such fragments are sufficient for use, for example, in immunodiagnostic procedures involving coupling the immunospecific portions of immunoglobulins to detecting reagents such as radiolabels. Alternatively, immunoglobulins and analogs with desired characteristics can be generated from immortalized B cells derived from the transgenic animals used in the method of the invention or from the rearranged genes provided by these animals in response to immunization. Thus, as an alternative to harvesting the antibodies directly from the animal, the B cells can be obtained, typically from the spleen, but also, if desired, from the peripheral blood lymphocytes or lymph nodes and immortalized using any of a variety of techniques, most commonly using the fusion methods described by Kohler and Milstein Nature 245:495 (1975). The resulting hybridomas (or otherwise immortalized B cells) can then be cultured as

single colonies and screened for secretion of antibodies of the desired specificity. As described above, the screen can also include a confirmation of the fully human character of the antibody. For example, as described in the examples below, a sandwich ELISA wherein the monoclonal in the hybridoma supernatant is bound both to antigen and to an antihuman constant region can be employed. After the appropriate hybridomas are selected, the desired antibodies can be recovered, again using conventional techniques. They can be prepared in quantity by culturing the immortalized B cells using conventional methods, either in vitro or in vivo to produce ascites fluid. Purification of the resulting monoclonal antibody preparations is less burdensome than in the case of serum since each immortalized colony will secrete only a single type of antibody. In any event, standard purification techniques to isolate the antibody from other proteins in the culture medium can be employed.

As an alternative to obtaining human immunoglobulins directly from the culture of immortalized B cells derived from the animal, the immortalized cells can be used as a source of rearranged heavy chain and light chain loci for subsequent expression and/or genetic manipulation. Rearranged antibody genes can be reverse transcribed from appropriate mRNAs to produce cDNA. If desired, the heavy chain constant region can be exchanged for that of a different isotype or eliminated altogether. The variable regions can be linked to encode single chain F<sub>2</sub> regions. Multiple F<sub>2</sub> regions can be linked to confer binding ability to more than one target or chimeric heavy and light chain combinations can be employed. Once the genetic material is available, design of analogs as described above which retain both the ability to bind the desired target, and their human characteristics, is straightforward.

Once the appropriate genetic material is obtained and, if desired, modified to encode an analog, the coding sequences, including those that encode, at a minimum, the variable regions of the human heavy and light chain, can be inserted into expression systems contained on vectors which

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can be transfected into standard recombinant host cells. As described below, a variety of such host cells may be used; for efficient processing, however, mammalian cells are preferred. Typical mammalian cell lines useful for this purpose include CHO cells, 293 cells, or NSO cells. The production of the antibody or analog is then undertaken by culturing the modified recombinant host under culture conditions appropriate for the growth of the host cells and the expression of the coding sequences. The antibodies are then recovered from the culture. The expression systems are preferably designed to include signal peptides so that the resulting antibodies are secreted into the medium; however, intracellular production is also possible.

In addition to deliberate design of modified forms of the immunoglobulin genes to produce analogs, advantage can be taken of phage display techniques to provide libraries containing a repertoire of antibodies with varying affinities for the desired antigen. For production of such repertoires, it is unnecessary to immortalize the B cells from the immunized animal; rather, the primary B cells can be used directly as a source of DNA. The mixture of cDNAs obtained from B cells, e.g., derived from spleens, is used to prepare an expression library, for example, a phage display library transduced into *E. coli*. The resulting cells are tested for immunoreactivity to the desired antigen. Techniques for the identification of high affinity human antibodies from such libraries are described by Griffiths, A.D., et al., *EMBO J.* (1994) 13:3245-3260; by Nisam, A., et al., *ibid*, 692-698, and by Griffiths, A.D., et al., *ibid*, 12:725-734. Ultimately, clones from the library are identified which produce binding affinities of a desired magnitude for the antigen, and the DNA encoding the product responsible for such binding is recovered and manipulated for standard recombinant expression. Phage display libraries may also be constructed using previously manipulated nucleotide sequences and screened in similar fashion. In general, the cDNAs encoding

heavy and light chain are independently supplied or are

The phage library is then screened for the

antibodies with highest affinity for the antigen and the

genetic material recovered from the appropriate clone.

Further rounds of screening can increase the affinity of the

original antibody isolated. The manipulations described

above for recombinant production of the antibody or

modification to form a desired analog can then be employed.

Combination of phage display technology with the

"Xenonouses" offers a significant advantage over previous

applications of phage display. Typically, to generate a

highly human antibody by phage display, a combinatorial

antibody library is prepared either from human bone marrow or

from peripheral blood lymphocytes as described by Burton,

D.R., et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:10134-

10137. Using this approach, it has been possible to isolate

high affinity antibodies to human pathogens from infected

individuals, i.e. from individuals who have been "immunized"

as described in Burton, D.R., et al., *Proc. Natl. Acad. Sci.*

*USA* (1991) 88:10134-10137, Zebde, S.T., et al., *Proc. Natl.*

*Acad. Sci. USA* (1992) 89:3175-3179, and Barbas III, C.F., et

al., *Proc. Natl. Acad. Sci. USA* (1991) 88:10164-20168.

However, to generate antibodies reactive with human antigens,

it has been necessary to generate synthetic libraries (Barbas

III C.F., et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:4457-

4461, Crameri, V. et al., *BioTechnology* (1995) 13:194-196) or

to prepare libraries from either autoimmune patients

(Kapoport, B., et al., *Immunol. Today* (1995) 16:43-49,

Portolano, S., et al., *J. Immunol.* (1993) 151:2839-2851, and

Vogel, M., et al., *Eur. J. Immunol.* (1994) 24:1200-1207) or

normal individuals, i.e. naive libraries (Griffiths, A.D., et

al., *EMBO J.* (1994) 13:3245-3260, Griffiths, A.D., et al.,

*Proc. Natl. Acad. Sci. USA* (1991) 88:2432-2436, Griffiths, A.D.,

Curt, Oph., *Immunol.* (1993) 8:263-267, Hoogenboom, H.R., et

al., *J. Mol. Biol.* (1992) 227:381-388, Lerner, R.A., et al.,

*Science* (1992) 258:1313-1314, and Nissim, A., et al., *EMBO J.*

(1994) 13:692-698. Typically, high affinity antibodies to human proteins have proven very difficult to isolate in this way. As is well known, affinity maturation requires somatic mutation and somatic mutation, in turn, is antigen driven. In the xenomouse, repeated immunization with human proteins will lead to somatic mutation and, consequently, high affinity antibodies. The genes encoding these antibodies can be readily amplified by PCR as described in Marks, J.D., et al., *J. Mol. Biol.* (1991) 581-596 and immunospecific antibodies isolated by standard panning techniques, Winter, G., et al., *Annu. Rev. Immunol.* (1994) 12:413-55 and Barbas III, C.F., et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:7978-7982.

As above, the modified or unmodified rearranged loci are manipulated using standard recombinant techniques by constructing expression systems operable in a desired host cell, such as, typically, a Chinese hamster ovary cell, and the desired immunoglobulin or analog is produced using standard recombinant expression techniques, and recovered and purified using conventional methods.

The application of the foregoing processes to antibody production has enabled the preparation of human immunospecific reagents with respect to antigens for which human antibodies have not heretofore been available. The immunoglobulins that result from the above-described methods and the analogs made possible thereby provide novel compositions for use in analysis, diagnosis, research, and therapy. The particular use will, of course, depend on the compositions of the invention will have utilities similar to those ascribable to nonhuman antibodies directed against the same antigen. Such utilities include, for example, use as affinity ligands for purification, as reagents in immunoassays, as components of immunocoujugates, and as therapeutic agents for appropriate indications.

Particularly in the case of therapeutic agents or diagnostic agents for use in vivo, it is highly advantageous to employ antibodies or their analogs with fully human

- characteristics. These reagents avoid the undesired immune responses engendered by antibodies or analogs which have characteristics marking them as originating from nonhuman species. Other attempts to "humanize" antibodies do not result in reagents with fully human characteristics. For example, chimeric antibodies with murine variable regions and human constant regions are easily prepared, but, of course, retain murine characteristics in the variable regions. Even the much more difficult procedure of "humanizing" the variable regions by manipulating the genes encoding the amino acid sequences that form the framework regions does not provide the desired result since the CDRs, typically of nonhuman origin, cannot be manipulated without destroying immunospecificity.
- Thus, the methods of the present invention provide, for the first time, immunoglobulins that are fully human or analogs which contain immunospecific regions with fully human characteristics.
- There are large numbers of antigens for which human antibodies and their human analogs would be made available by the methods of the invention. These include, but are not limited to, the following nonlimiting set:
- leukocyte markers, such as CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD11a,b,c, CD13, CD14, CD18, CD19, CD20, CD22, CD23, CD27 and its ligand, CD28 and its ligands B7.1, B7.2, B7.3, CD29 and its ligand, CD30 and its ligand, CD40 and its ligand gp39, CD44, CD45 and isoforms, CD452 (Campath antigen), CD56, CD58, CD69, CD72, CTLA-4, LFA-1 and TCR
- histocompatibility antigens, such as MHC class I or II, the Lewis x antigens, Slex, Sley, Slea, and Selb;
- adhesion molecules, including the integrins, such as VLA-1, VLA-2, VLA-3, VLA-4, VLA-5, VLA-6, LFA-1, Mac-1, αVβ3, and p150,95; and



the selectins, such as L-selectin, E-selectin, and P-selectin and their counterreceptors VCAM-1, ICAM-1, and LFA-3;

interleukins, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, and IL-15;

interleukin receptors, such as IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-8R, IL-9R, IL-10R, IL-11R, IL-12R, IL-13R, IL-14R and IL-15R;

chemokines, such as PF4, RANTES, MIP1 $\alpha$ , MCP1, IP-10, ENA-78, NAP-2, GRO $\alpha$ , GRO $\beta$ , and IL-8;

growth factors, such as TNF $\alpha$ , TNF $\beta$ , TGF $\beta$ , TSH, VEGF/VEGF, PDGF family, FGF, PDGF family, endothelin, fibronectin (F $\beta$ ), laminin, and gastrin releasing peptide (GRP);

growth factor receptors, such as TNF $\alpha$ , TNF $\beta$ , TSHR, VEGFR/VEGF, PDGF, PDGFR, PDGFR family, EGF-R, GCSF-R and other hematopoietic receptors;

interferon receptors, such as IFN $\alpha$ , IFN $\beta$ , and IFN $\gamma$ ;

IgE and their receptors, such as IgE, Fc $\epsilon$ R1, and Fc $\epsilon$ R2;

tumor antigens, such as her2-neu, mucin, CEA and endostatin;

allergens, such as house dust mite antigen, 101 p1 (grass) antigens, and urushiol;

viral proteins, such as CMV glycoproteins B, H, and gCIII, HIV-1 envelope glycoproteins, RSV envelope

- glycoproteins, HSV envelope glycoproteins, EBV envelope glycoproteins, VZV, envelope glycoproteins, HIV envelope glycoproteins, Hepatitis family surface antigens;
- 5 toxins, such as pseudomonas endotoxin and osteopontin/urapontin, snake venom, spider venom, and bee venom;
- 10 blood factors, such as complement C3b, complement C5a, complement C5b-9, Rh factor, fibrinogen, fibrin, and myelin associated growth inhibitor;
- enzymes, such as cholesterol ester transfer protein, membrane bound matrix metalloproteinases, and glutamic acid decarboxylase (GAD); and
- 15 miscellaneous antigens including ganglioside GD3, ganglioside GM2, LMP1, LMP2, eosinophil major basic protein, PTHrP, eosinophil cationic protein, pANCA, vimentin protein, Type IV collagen, glycosylated lipids,  $\alpha$ -interferon,  $\gamma$ 1, p-glycoprotein and Fas (Apo-1) and oxidized-LDL.
- Particularly preferred immunoglobulins and analogs are those immunospecific with respect to human IL-6, human IL-8, human TNF $\alpha$ , human CD4, human L-selectin, human PTHrP and human gp39. Antibodies and analogs immunoreactive with human TNF $\alpha$  and human IL-6 are useful in treating cachexia and septic shock as well as autoimmune disease. Antibodies and analogs immunoreactive with gp39 or with L-selectin are also effective in treating or preventing autoimmune disease. In addition, anti-gp39 is helpful in treating graft versus host disease, in preventing organ transplant rejection, and in treating glomerulonephritis. Antibodies and analogs against L-selectin are useful in treating ischemia associated with reperfusion injury. Antibodies to PTHrP are useful in treating bone disease and metastatic cancer. In a particular embodiment, human antibodies against IL-8 may be used for the treatment or prevention of a pathology or condition

associated with II-8. Such conditions include, but are not limited to, tumor metastasis, reperfusion injury, pulmonary edema, asthma, ischemic disease such as myocardial infarction, inflammatory bowel disease (such as Crohn's disease and ulcerative colitis), encephalitis, uveitis, autoimmune diseases (such as rheumatoid arthritis, Sjögren's syndrome, vasculitis), osteoarthritis, gouty arthritis, nephritis, renal failure, dermatological conditions such as inflammatory dermatitis, psoriasis, vasculitic ulcers and allergic angitis, retinal uveitis, conjunctivitis,

neurological disorders such as stroke, multiple sclerosis and meningitis, acute lung injury, adult respiratory distress syndrome (ARDS), septic shock, bacterial pneumonia, diseases involving leukocyte diapedesis, CNS inflammatory disorder, multiple organ failure, alcoholic hepatitis, antigen-antibody complex mediated diseases, inflammation of the lung (such as pleurisy, abscesses, vasculitis, pneumonia, chronic bronchitis, bronchiectasis, cystic fibrosis), Behcet disease, Wegener's granulomatosis, and vasculitic syndrome.

Typical autoimmune diseases which can be treated using the above-mentioned antibodies and analogs include systemic lupus erythematosus, rheumatoid arthritis, psoriasis, Sjögren's scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, Reiter's syndrome, Behcet's disease, Type 1 diabetes, Hashimoto's thyroiditis, Grave's disease, multiple sclerosis, myasthenia gravis and pemphigus.

For therapeutic applications, the antibodies may be administered in a pharmaceutically acceptable dosage form. They may be administered by any means that enables the active agent to reach the desired site of action, for example, intravenously as by bolus or by continuous infusion over a period of time, by intramuscular, subcutaneous, intrascicular, intrathecal, intracavitary, topical or inhalation routes. The antibodies may be administered as a single dose or a series of treatments.

For parenteral administration, the antibodies may be formulated as a solution, suspension, emulsion or

using plate coating buffer (0.1 M carbonate buffer, pH 9.6) ;

Unless otherwise noted, the antigen was coated  
microplate reader.

the plates were read 30 minutes later at 492 nm using a  
35 diamine (OPD) substrate and hydrogen peroxide were added and  
After again washing, the chromogenic reagent o-phenylene  
conjugated to horseradish peroxidase (HRP) for one hour.

30 detected by adding antihuman  $\kappa$ ,  $\mu$ , or  $\gamma$  chain antibody  
washing, bound antibody having human characteristics was  
as serial dilutions for 1-2 hours of incubation. After  
washed and blocked and the sera (or supernatants) were added  
immobilized onto wells of microtiter plates. The plates were

25 In this format, the antigen used for immunization was  
human antibodies in each case using a standard ELISA format.  
from immortalized B cells) were titrated for antigen specific  
The sera of the immunized xenomouse" (or the supernatants  
to each antigen are described in the specific examples below.

20 application No 94/02602. Immunization protocols appropriate  
the xenomouse" is found in the above referenced PCT  
used for initial immunizations. A detailed description of  
In these examples, mice, designated xenomouse", are

15 intended to illustrate but not to limit the invention.  
physiological effect desired. The examples below are  
frequency of treatment, concurrent treatment and the

10 be treated and the severity and course of the condition,  
weight, and health of the recipient, the type of condition to  
antibody, its mode and route of administration, the age,  
such as the pharmacodynamic characteristics of the particular  
appropriate dosage of antibody will depend upon known factors

For prevention or treatment of disease, the  
reference text in this field.

5 Remington's Pharmaceutical Sciences, V. Osol, a standard  
suitable vehicles are described in the most recent edition of  
various sugars, magnesium carbonate, or calcium phosphate.  
additives such as, for example, starch, cellulose, silica,  
acceptable parenteral vehicle. If the antibody is suitable  
lyophilized powder in association with a pharmaceutically

the assay blocking buffer used was 0.5% BSA, 0.1% Tween 20 and 0.01% thimerosal in PBS; the substrate buffer used in color development was citric acid 7.14 g/l; dibasic sodium phosphate 17.96 g/l; the developing solution (made immediately before use) was 10 ml substrate buffer; 10 mg OPD, plus 5 ml hydrogen peroxide; the stop solution (used to stop color development) was 2 M sulfuric acid. The wash solution was 0.05% Tween 20 in PBS.

#### Example 1

**Human Antibodies Against Human IL-6**

Three to five "Xenomouse" aged 8-20 weeks were age-matched and immunized intraperitoneally with 50 µg human IL-6 emulsified in incomplete Freund's adjuvant for primary immunization and in complete Freund's adjuvant for subsequent injections. The mice received 6 injections 2-3 weeks apart. Serum titers were determined after the second dose and following each dose thereafter. Bloods were performed from the retrobulbar plexus 6-7 days after injections. The blood was allowed to clot at room temperature for about 2 hours and then incubated at 4°C for at least 2 hours before separating and collecting the sera.

ELISAs were conducted as described above by applying 100 µl/well of recombinant human IL-6 at 2 µg/ml in coating buffer. Plates were then incubated at 4°C overnight or at 37°C for 2 hours and then washed three times in washing buffer. Addition of 100 µl/well blocking buffer was followed by incubation at room temperature for 2 hours, and an additional 3 washes.

Then, 50 µl/well of diluted serum samples (and positive and negative controls) were added to the plates. Plates were then incubated at room temperature for 2 hours and again washed 3 times.

After washing, 100 µl/well of either mouse anti-human  $\mu$  chain antibody conjugated to HRP at 1/2,000 or mouse anti-human  $\kappa$  chain antibody conjugated to HRP at 1/2,000, diluted in blocking buffer was added. After a 1

hour incubation at room temperature, the plates were washed 3 times and developed with OPD substrate for 10-25 minutes. 50  $\mu$ l/well of stop solution was then added and the results read on an ELISA plate reader at 492 nm. The dilution curves resulting from the titration of serum from "Xenomouse" after 6 injections are shown in Figure 3. The data in Figure 3 show production of anti-IL-6 immunoreactive with anti-human  $\kappa$  and antihuman  $\mu$  detectable at serum dilutions above 1:1,000.

**Example 2**

**Human Antibodies Against Human TNF $\alpha$**

Immunization and serum preparation were conducted as described in Example 1 except that human recombinant TNF $\alpha$  (at 5  $\mu$ g per injection) was substituted for human IL-6. ELISAs were conducted as described in Example 1 except that the initial coating of the ELISA plate employed 100  $\mu$ l/well recombinant human TNF $\alpha$  at 1  $\mu$ g/ml in coating buffer. The dilution curves for serum from "Xenomouse" after 6 inductions obtained are shown in Figure 4. Again significant titers of human anti-TNF $\alpha$  binding were shown. Serum titers for h $\gamma$ , h $\mu$ , and h $\kappa$  after one and two immunizations of the "Xenomouse" are shown in Table 1. When challenged with TNF- $\alpha$ , the "Xenomouse" switches isotypes from a predominant IgM response in the first immunization to an immune response with a large IgG component in the second immunization.

TABLE 2. Anti TNF-alpha serum titer responses of Xenomouse-2.

Bleed 1: after 2 immunizations  
Bleed 2: after 3 immunizations

ELISA		Xm2			
Serum titers					
Specific for TNF-alpha					
titer		titer		titer	
(via hr)		(via hr)		(via hr)	
1,500	3,000	500	10,000	bleed 1	1
15,000	8,000	200	2,700	bleed 2	2
500	3,000	<500	15,000	bleed 1	3
1,000	5,000	500	70,000	bleed 2	4
1,500	2,500	1,000	1,000	bleed 1	5
72,000	4,000	2,500	2,500	bleed 2	6
1,500	2,500	13,000	4,500	bleed 1	7
7,000	10,000	2,700	5,000	bleed 2	8
4,000	6,000	200	40,000	bleed 1	9
80,000	80,000	200	15,000	bleed 2	10
500	2,000	1,500	24,000	bleed 1	11
60,000	8,000	200	2,000	bleed 2	12
1,500	1,000	2,000	10,000	bleed 1	13
72,000	2,700	500	2,000	bleed 2	
25,000	4,000	30,000	4,000		
12,000	4,000	500	2,000		

Human antibodies Against Human CD4  
Example 3

The human CD4 antigen was prepared as a surface protein using human CD4<sup>+</sup> on transfected recombinant cells as follows. Human CD4<sup>+</sup> consists of the extracellular domain of CD4, the transmembrane domain of CD4, and the cytoplasmic domain corresponding to residues 31-142, of the mature chain of the CD3 complex. Human CD4 zeta (F15 I/R) as described in Roberts et al., Blood (1994) 84:2878 was introduced into the rat basophil leukemic cell line RBL-2H3, described by Callan, M., et al., Proc Natl Acad Sci USA (1993) 90:10454 using the *Kat* high efficiency transduction described by Finer et al., Blood (1994) 83:43. Briefly, RBL-2H3 cells at  $10^6$  cells per well were cultured in 750  $\mu$ l DMEM + 20% FBS (Gibco) and 16  $\mu$ g/ml polybrene with an equal volume of preplated supernatant for 2 hours at 37°C, 5% CO<sub>2</sub>. One ml of medium was removed and 750  $\mu$ l of infection medium and retroviral supernatant were added to each well and the cultures incubated overnight. The cells were washed and expanded in DMEM + 10% FBS until sufficient cells were available for sorting. The CD4 zeta transduced RBL-2H3 cells were sorted using the FACSTAR plus (Becton Dickinson). The cells were stained for human CD4 with a mouse anti-human CD4 PE antibody and the top 2-3% expressing cells were selected. Immunizations were conducted as described in Example 1 using  $1 \times 10^7$  cells per mouse except that the primary injection was subcutaneous at the base of the neck. The mice received 6 injections 2-3 weeks apart. Serum was prepared and analyzed by ELISA as described in Example 1 except that the initial coating of the ELISA plate utilized 100  $\mu$ l per well of recombinant soluble CD4 at 2  $\mu$ g/ml of coating buffer. The titration curve for serum from "Xenomouse" after 6 injections is shown in Figure 5. Titers of human anti-CD4 reactively were shown at concentrations representing greater than those of 1:1,000 dilution.



#### Example 4

##### Human Antibodies Against Human L-selectin

The antigen was prepared as a surface displayed protein in CHO cells, a high expressing clone derived by

transfecting the mouse pre-B cell 300.19 with hL-1 cDNA (L-1 is the gene encoding L-selectin) (Tedder, et al., J. Immunol. (1990) 144:532) or with similarly transfected CHO

cells. The transfected cells were sorted using fluorescent activated cell sorting using anti-Leu-8 antibody as label.

The CHO and the transfected CHO cells were grown in DMF 4.5 g/l glucose with 10% FCS and 1 mg/ml G418 in 100 mm

dishes. Negative control cells, 3T3-P317 (transfected with gag/pol/nev genes of Moloney virus) were grown in the same

medium without G418.

Primary immunization was done by injection subcutaneously at the base of the neck; subsequent injections

were intraperitoneal. 70-100 million CS1 or transfected CHO cells were used per injection for a total of five injections

2-3 weeks apart.

Sera were collected as described in Example 1 and analyzed by ELISA in a protocol similar to that set forth in

Example 1.

For the ELISA, the transfected cells were plated into 96 well plates and cell monolayers grown for 1-2 days

depending on cell number and used for ELISA when confluent. The cells were fixed by first washing with cold 1 x PBS and

then fixing solution (5% glacial acetic acid, 95% ethanol) was added. The plates were incubated at -25°C for 5 minutes

and can be stored at this temperature if sealed with plate

sealers. The ELISA is begun by bringing the plates to room temperature, flicking to remove fixing solution and washing 5

times with DMEM medium containing 10% FCS at 200 µl per well. The wells were treated with various serum dilutions

or with positive or negative controls. Positive control wells contained murine IgG1 monoclonal antibody to human L-selectin.

The wells were incubated for 45 minutes and monolayer integrity was checked under a microscope. The wells were then incubated with anti-human  $\alpha$  chain antibody or anti-human  $\beta$  chain antibody conjugates with HRP described in Example 1. The plates were then washed with 1% BSA/PBS and again with PBS and monolayer integrity was checked. The plates were developed, stopped, and read as described above. The results for serum from Xenomouse<sup>®</sup> are shown in Figures 6 and 7; human antibodies both to L-selectin and control J73 cells were obtained. However, the serum titers are higher for the L-selectin-expressing cells as compared to parental J73 cells. These results show the Xenomouse<sup>®</sup> produces antibodies specific for L-selectin with human  $\mu$  heavy chain regions and human  $\kappa$  light chains.

The antisera obtained from the immunized Xenomouse<sup>®</sup> were also tested for staining of human neutrophils which express L-selectin. Human neutrophils were prepared as follows:

peripheral blood was collected from normal volunteers with 100 units/ml heparin. About 3.5 ml blood was layered over an equal volume of one-step polymorph gradient (Accurate Chemical, Westbury, NY) and spun for 30 minutes at 450 x g at 20°C. The neutrophil fraction was removed and washed twice in DPBS/2% FBS.

The neutrophils were then stained with either:

(1) antiserum from Xenomouse<sup>®</sup> immunized with C51 cells (expressing L-selectin);

(2) as a negative control, antiserum from a Xenomouse<sup>®</sup> immunized with cells expressing human gp39.

The stained, washed neutrophils were analyzed by FACS. The results for antiserum from Xenomouse<sup>®</sup> are shown in Figure 8.

These results show the presence of antibodies in immunized Xenomouse<sup>®</sup> serum which contain fully human light chains immunoreactive with L-selectin. The negative control

35  
30  
25  
15  
10  
5

antiserum from mice immunized with gp39 does not contain antibodies reactive against human neutrophils.

Example 5

Human Antibodies Against Human gp39

gp39 (the ligand for CD40) is expressed on activated human CD4<sup>+</sup> T cells. The sera of "Xenohouse" immunized with recombinant gp39 according to this example contained fully human antibodies immunospecific for gp39. The antigen consisted of stable transfectants of 300.19 cells or of CHO cells expressing gp39 cDNA cloned into the mammalian expression vector pRK1.HUGp39/INRES NEO as shown in Figure 9. CHO cells were split 1:10 prior to transfection in DMEM 4.5 g/l glucose, 10% FBS, 2 mM glutamine, MEM, NEAA supplemented with additional glycine, hypoxanthine and thymidine. The cells were cotransfected with the gp39 vector at 9 µg/10 cm plate (6 x 10<sup>6</sup> cells) and the DHFR expressing vector pSV2DHFRs (Subramani et al., Mol Cell Biol (1981) 2:854) at 1 µg/10 cm plate using calcium phosphate transfection. 24 hours later the cells were split 1:10 into the original medium containing G418 at 0.6 mg/ml. Cells producing gp39 were sorted by FACS using an anti-gp39 antibody.

Mice grouped as described in Example 1 were immunized with 300.19 cells expressing gp39 using primary immunization subcutaneously at the base of the neck and with secondary intraperitoneal injections every 2-3 weeks. Sera were harvested as described in Example 1 for the ELISA assay. The ELISA procedure was conducted substantially as set forth in Example 1; the microtiter plates were coated with CHO cells expressing gp39 grown in a 100 mm dish in DMEM, 4.5 g/l glucose, 10% FBS, 4 mM glutamine, and nonessential amino acid (NEAA) solution for MEM (100X). On the day preceding the ELISA assay, the cells were trypsinized and plated into well filtration plates at 10<sup>6</sup> cells/200 µl well and incubated at 37°C overnight. The positive controls were mouse anti-human gp39; negative controls were antisera from mice immunized with an antigen other than gp39. 50 µl of sample were used

for each assay. The remainder of the assay is as described in Example 1.

The dilution curves for the sera obtained after 4

injections from mice immunized with gp39 expressed on CHO

cells are shown in Figure 10. As shown, the sera contained

antihuman gp39 immunospecificity which is detectable with

anti-human  $\kappa$  and anti-human  $\mu$  chain antibodies coupled to

HRP.

#### Example 6

#### Preparation of Human Mabs Against Tetanus Toxin

The antibodies prepared in this example were

secreted by hybridomas obtained by immortalizing B cells from

xenotice immunized with tetanus toxin. The immunization

protocol was similar to that set forth in Example 1 using 50

$\mu$ g tetanus toxin emulsified in complete Freund's adjuvant for

interprimeal primary immunization followed by subsequent

interprimeal injections with antigen incorporated into

incomplete Freund's adjuvant. The mice received a total of 4

injections 2-3 weeks apart.

After acceptable serum titers of antitetanus toxin

C (anti-TTC) were obtained, a final immunization dose of

antigen in PBS was given 4 days before the animals were

sacrificed and the spleens were harvested for fusion.

The spleen cells were fused with myeloma cells

P3X63-Ag8.653 as described by Galfré, G. and Milstein, C.

Methods in Enzymology (1981) 73:3-46.

After fusion the cells were resuspended in DMEM,

15% FCS, containing HAT supplemented with glutamine,

pen/strep for culture at 37°C and 10% CO<sub>2</sub>. The cells were

plated in microtiter plates and maintained in HAT-

supplemented medium for two weeks before transfer to HAT-

supplemented medium. Supernatants from wells containing

hybridomas were collected for a primary screen using an

ELISA.

The ELISA was conducted as described in Example 1

wherein the antigen coating consisted of 100  $\mu$ l/well of

tetanus toxin C (TTC) protein at 2  $\mu$ g/ml in coating buffer.

followed by incubation at 4°C overnight or at 37°C for two hours. In the primary ELISA, HRP-conjugated mouse anti-human IgM was used as described in Example 1. Two hybridomas that secreted anti-ITC according to the ELISA assay, clone D5.1 and clone K4.1 were used for further analysis.

As shown in Figure 11, clone D5.1 secretes fully human anti-ITC which is detectable using HRP-conjugated anti-human  $\mu$  chain antibody and HRP-conjugated anti-human  $\kappa$  chain antibody. This is confirmed in Figure 11.

The antibody secreted by D5.1 did not immunoreact in ELISAs using TNF $\alpha$ , IL-6, or IL-8 as immobilized antigen under conditions where positive controls (sera from xenomice immunized with TNF $\alpha$ , IL-6 and IL-8 respectively) showed positive ELISA results.

The complete nucleotide sequence of the cDNAs encoding the heavy and light chains of the monoclonal were determined as shown in Figures 12 and 13. polyA mRNA was isolated from about 10<sup>6</sup> hybridoma cells and used to generate cDNA using random hexamers as primers. Portions of the product were amplified by PCR using the appropriate primers.

The cell line was known to provide human  $\kappa$  light chains; for PCR amplification of light chain encoding cDNA, the primers used were HKP1 (5'-CTGTGACACACTCTCTGGAGATT-3') for priming from the constant region terminus and two oligos, used in equal amounts to prime from the variable segments; B3 (5'-GAAACGACACACTCAACGACCTCCAGC-3').

For amplification of the heavy chain of the antibody derived from D5.1 (which contains the human  $\mu$  constant region), MG-24VI was used to prime from the variable and  $\mu$ P1 (5'-TTTCTTTGTTGCCCCGTTGGGTGC-3') was used to prime from the constant region terminus.

Referring to Figure 12 which sets forth the sequence for the heavy chain of the antibody secreted by clone D5.1, this shows the heavy chain is comprised of the human variable fragment VH6, the human diversity region DNI and the human joining segment JH4 linked to the human  $\mu$  constant region. There were two base-pair mutations from the germline sequence in the variable region, both in the CDRs.

Two additional mutations were in the D segment and six nongermline nucleotide additions were present at the D-J junction. Finally, referring to Figure 13 which presents the light chain of the antibody secreted by D5.1, the human variable region B3 and human  $\kappa$  joining region JX3 are shown. There are nine base-pair differences from the germline sequences, three falling with CDR1.

Example 7

Groups of Xenomouse<sup>TM</sup>-2 were immunized intraperitoneally with either PTHrP (1-34) conjugated with B7G, as described by Ratcliffe et al., J. Immunol. Methods 132:109 (1990), or with PTHrP (1-34) synthesized as a branched-KAP (multiple antigenic peptide system). The antigens were emulsified in CFA (complete Freund's adjuvant) and injected i.p. at a dose of 25  $\mu$ g per animal at 2 week intervals, and bled after two injections. The sera obtained from this bleed were analyzed by ELISA as described supra. Serum titers for h $\gamma$ , h $\mu$ , and h $\kappa$  after one immunization of the Xenomouse<sup>TM</sup> are shown in Table 2. When immunized with PTHrP, the Xenomouse<sup>TM</sup> showed low serum titers in 5 of 7 mice on the first bleed, but when PTHrP-KAP is used, 7 of 7 mice show high serum titers on the first bleed.

ELISA assays were performed with respect to the recovered serum, also exactly as described in Example 1, except that the ELISA plates were initially coated using 100  $\mu$ l/well of recombinant human IL-8 at 0.5mg/ml in the coating buffer. The results obtained for various serum dilutions from Xenomouse<sup>®</sup> after 6 injections are shown in Figure 14.

used as an immunogen.

described in Example 1 except that human recombinant IL-8 was immunization and serum preparation were as

Human Antibodies Against Human IL-8

Example 8

Human Responses			
Xm2		PTHrP-BTG	PTHrP-MAP
titers	(via hr)	titers	(via hr)
1	<30	1	<30
2	<30	2	<30
3	<30	3	<30
4	<30	4	<30
5	<30	5	<30
6	<30	6	<30
7	<30	7	<30
100	850		
50	3,000		
1,000	7,000		
200	800		
90	400		
50	500		
50	300		
1	<30	1	<30
2	<30	2	<30
3	<30	3	<30
4	<30	4	<30
5	<30	5	<30
6	<30	6	<30
7	<30	7	<30
150	1,000		
300	2,500		
270	1,000		
300	2,500		
150	1,200		
150	1,000		
800	4,000		

TABLE 1. AntiPTHrP serum titer responses of Xenomouse-2.

First bleed after 2 immunizations with either PTHrP-BTG conjugate

Human anti-IL-8 binding was again shown at serum dilutions having concentrations higher than that represented by a 1:1,000 dilution.

### Example 2

#### Preparation of High Affinity Human Monoclonal Antibodies Against Human IL-8

Groups of 4 to 6 Xenomouse<sup>®</sup> aged between 8 to 10

weeks old were used for immunization and for hybridoma

generation. Xenomouse<sup>®</sup> were immunized intraperitoneally with

25 µg of human recombinant-IL-8 (Biosource International, CA,

USA) emulsified in complete Freund's adjuvant (CFA, Sigma)

for the primary immunization. All subsequent injections were

done with the antigen incorporated into incomplete Freund's

adjuvant (IFA, Sigma). For animals used as spleen donors for

hybridoma generation a final dose of antigen in phosphate

buffer saline (PBS) was given 4 days before the fusion.

Serum titers of immunized Xenomouse<sup>®</sup> were first analyzed

after a secondary dose of antigens, and from there after,

following every antigen dose. Test bleeds were performed 6

to 7 days after the injections, by bleeding from the retro-

bulbar plexus. Blood was allowed to clot at room temperature

for about 2 hours and then incubated at 4°C for at least 2

hours before separating and collecting the sera.

#### Generation of hybridomas

Spleen cells obtained from Xenomouse<sup>®</sup> previously

immunized with antigen, were fused with the non secretory NSO

myeloma cells transfected with pc1-2 (NSO-pc12) as described

in Galfré G, et al., Methods in Enzymology 73, 3-46, (1981).

Briefly, the fusion was performed by mixing washed spleen

cells and myeloma cells at a ratio of 5:1 and gently

pelletting them by centrifugation at 800Xg. After complete

removal of the supernatant the cells were treated with 1 ml

of 50% PBS/DMSO (polyethylene glycol MW 1500, 10% DMSO,

Sigma) which was added over 1 min., the mixture was further

incubated for one minute, and gradually diluted with 2 ml of

DMSO over 2 minutes and diluted further with 8 ml of DMSO



over 3 minutes. The process was performed at 37 °C with continued gentle stirring. After fusion the cells were resuspended in DMEM, 15% FCS, containing HAT, and supplemented with 1 glutamine, pen/strep, for culture at 37 °C and 10% CO<sub>2</sub> in air. Cells were plated in flat bottomed 96 well microtiter trays. Cultures were maintained in HAT supplemented media for 2 weeks before transfer to HT hybrid cell growth, and supernatants were regularly examined for containing hybridomas were collected for a primary screen analysis for the presence of human  $\mu$ , human gamma 2, and human kappa chains in an antigen specific ELISA as described above. Positive cultures were transferred to 48 well plates and when reaching confluence transferred to 24 well plates. Supernatants were tested in an antigen specific ELISA for the presence of human  $\mu$ , human gamma 2, and human kappa chains. As shown in Table 3 several hybridomas secreting fully human monoclonal antibodies with specificity for human IL-8 have been generated from representative fusions. In all of these human monoclonal antibodies the human gamma-2 heavy chain is associated with the human kappa light chain.

TABLE 3: ELISA determination of heavy and light chain composition of anti-IL-8 human monoclonal antibodies generated in Xenomouse™

Sample	ID	Ig class	titers	reactivity to hIL8				Total IgG (ng/ml)
				H <sub>e</sub> OD (1:1)	m <sub>a</sub> OD (1:1)	h <sub>y</sub> OD (1:1)		
					0.08	0.04	0.12	
					4.12	0.04	4.09	1.159
					200	4.18	0.18	4.11
					1,000	4.00	0.04	4.583
					200	3.98	0.04	3.49
					200	3.80	0.05	4.09
					1,000	4.00	0.06	4.00
					1,468			

Evaluation of kinetic constants of Xenomouse™ hybridomas in order to determine the kinetic parameters of these antibodies, specifically their on and off rates and their dissociation constants (K<sub>D</sub>), they were analyzed on the BIAcore instrument (Pharmacia). The BIAcore instrument uses plasmon resonance to measure the binding of an antibody to an antigen-coated gold chip.

BIAcore reagents and instrumentation:

The BIAcore instrument, CMS sensor chips, surfactant P20, and the amine coupling kit containing N-hydroxysuccinimide (NHS), N-ethyl-N-(3-dimethylaminopropyl)-carbodiimide (EDC), and ethanolamine were purchased from

Pharmacia Biosensor. Immobilization of human recombinant IL-8 onto the sensor surface was carried out at low levels of antigen density immobilized on the surface and was performed according to the general procedures outlined by the manufacturers. Briefly, after washing and equilibrating the instrument with HEPES buffer (HBS; 10 mM HEPES, 150 mM NaCl, 0.05% surfactant P20, pH 7.4) the surface was activated and IL-8 immobilized for the subsequent binding and kinetic studies. The sensor surface was activated with 5  $\mu$ l of a mixture of equal volumes of NHS (0.1 M) and EDC (0.1 M) injected at 10  $\mu$ l/min across the surface for activation, then 5  $\mu$ l of the ligand (human recombinant IL-8) at 12  $\mu$ g/ml in 5 mM maleate buffer, pH 6.0 was injected across the activated surface, and finally non-conjugated active sites were blocked with an injection of 35  $\mu$ l of 1 M ethanolamine. The surface was washed to remove non-covalently bound ligand by injection of 5  $\mu$ l 0.1 M HCl. All the immobilization procedure was carried out with a continuous flow of HBS at 10  $\mu$ l/min. About 100 resonance units (RU) of ligand (82 and 139 RU, in separate experiments) were immobilized on the sensorship, (according to the manufacturers 1,000 RU corresponds to about 1 ng/mm<sup>2</sup> of immobilized protein). These ligand coated surfaces were used to analyze hybridoma supernatants for their specific binding to ligand and for kinetic studies. The best regenerating condition for the analyte dissociation from the ligand in these sensorships was an injection of 10  $\mu$ l 100 mM HCl with no significant losses of binding observed after many cycles of binding and regeneration.

Determination of the dissociation, and association rates and the apparent affinity constants of fully human monoclonal antibodies specific for IL-8. The determination of kinetic measurements using the BIAcore in which one of the reactants is immobilized on the sensor surface was done following procedures suggested by the manufacturers and described in Karlsson et al. "Kinetic analysis of monoclonal antibody-antigen interaction with a

new biosensor based analytical system." J. Immunol. Methods (1991) 145, 229. Briefly the single site interaction between two molecules A and B is described by the following equation.

$$d[AB]/dt = k_a[A][B] - k_d[AB]$$

In which B is immobilized on the surface and A is injected at a constant concentration C. The response is a measure of the concentration of the complex [AB] and all concentration terms can be expressed as Response Units (RU) of the BIAcore:

$$dR/dt = k_a C(R_{max} - R) - k_d R$$

where  $dR/dt$  is the rate of change of the signal, C is the

concentration of the analyte,  $R_{max}$  is the maximum analyte

binding capacity in RU and R is the signal in RU at time t.

In this analysis the values of  $k_a$  and  $k_d$  are independent of

the concentration of immobilized ligand on the surface of the

sensor. The dissociation rates ( $k_d$ ) and association rates

( $k_a$ ) were determined using the software provided by the

manufacturers, BIA evaluation 2.1. The dissociation rate

constant was measured during the dissociation phase that

extended for 10 minutes at a constant buffer flow rate of 45

$\mu$ l/min, after the completion of the injection of the

hybridoma supernatants onto the surface containing

immobilized IL-8. The association phase extended over 1.25

minutes at a flow rate of 45  $\mu$ l/min and the data was fitted

into the model using the previously determined  $k_d$  values. At

least two surfaces with different levels of immobilized

ligand were used in which different concentrations of anti

IL-8 hybridoma supernatants were tested for binding and

analyzed for kinetic data. The kinetic constants determined

on these two surfaces are presented in Table 4. The

affinities were determined to be very, ranging from  $7 \times 10^{11}$

to  $2 \times 10^7$  M. This compares very favorably with the

affinities of murine monoclonal antibodies derived from

normal mice.

TABLE 4: Kinetic constants of fully human monoclonal antibodies (lgG2, kappa) derived from Xenomouse™ II-a with specificity to human IL-8, determined by BIAcore.

Hybridoma	$k_a$ ( $M^{-1}s^{-1}$ )	dissociation rate $k_d$ ( $s^{-1}$ )	Dissociation Constant $KD$ ( $M$ )= $k_d/k_a$	BIAcore surface n-IL-8 [RU]
18D1-1	$3.36 \times 10^6$	$2.58 \times 10^{-4}$	$7.70 \times 10^{-11}$	81
18K2-1	$4.38 \times 10^5$	$6.73 \times 10^{-4}$	$1.54 \times 10^{-9}$	81
18K2-2	$5.24 \times 10^5$	$2.26 \times 10^{-4}$	$4.30 \times 10^{-10}$	81
18K4-2	$5.76 \times 10^6$	$8.17 \times 10^{-4}$	$1.42 \times 10^{-10}$	81
18K4-3	$2.66 \times 10^6$	$7.53 \times 10^{-4}$	$2.83 \times 10^{-10}$	81
18K4-5	$4.00 \times 10^5$	$9.04 \times 10^{-4}$	$2.26 \times 10^{-9}$	81

Methods for isolation of human neutrophils and assays for antibody activity

The primary *in vivo* function of IL-8 is to attract and activate neutrophils. Neutrophils express on their surface two distinct receptors for IL-8, designated the A receptor and the B receptor. In order to determine whether the fully human antibodies could neutralize the activity of IL-8, two different *in vitro* assays were performed with human neutrophils. In one assay, the ability of the antibodies to block binding or radiolabelled IL-8 to neutrophil IL-8 receptors was tested. In a second assay, the antibodies were tested for their ability to block an IL-8-induced

neutrophil response, namely the upregulation of the integrin Mac-1 on the neutrophil surface. Mac-1 is composed of two polypeptide chains, CD11b and CD18. Typically, anti-CD11b antibodies are used for its detection.

#### Isolation of neutrophils:

Human neutrophils are isolated from either freshly drawn blood or buffy coat. Human blood is collected by

ventipuncture into sterile tubes containing EDTA. Buffy coats are obtained from Stanford Blood Bank. They are prepared by centrifuging anticoagulated blood (up to 400 ml) in plastic bags at 2600 xg for 10 min at 20°C with the brake off. The plasma supernatant is aspirated out of the bag and the buffy coat, i.e., the upper cell layer (40-50 ml/bag) is collected. One unit of buffy coat (40-50 ml) is diluted to final volume of 120 ml with Ca<sup>2+</sup>, Mg<sup>2+</sup>-free PBS. 30 milliliters of blood or diluted buffy coat are transferred into 50-ml centrifuge tubes on top of a 20-ml layer of Ficoll-Paque Plus (Pharmacia Biotech). The tubes are centrifuged at 500 xg for 20 min at 20°C with brake off. The supernatant, the mononuclear cells, the cell pellet containing neutrophils and

erythrocytes is resuspended with 5 ml of PBS and transferred into clean 50-ml tubes. The cells are washed in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free PBS (300 xg for 5 min at 4°C). The erythrocytes are then lysed with ammonium chloride. The cells are resuspended in 40 ml of an ice-cold solution containing 155 mM NH<sub>4</sub>Cl and 10 mM EDTA, pH 7.2-7.4. The tubes are kept on ice for 10 min with occasional mixing and then centrifuged at 300 xg for 5 min at 4°C. The pellet is resuspended in PBS and washed once (300 xg for 5 min at 4°C). If erythrocyte lysis appears incomplete, the treatment with ammonium chloride is repeated.

The neutrophils are again washed and finally suspended either in assay medium (RPMI-1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 5x10<sup>-5</sup> 2-mercaptoethanol, 1X non-essential amino acids, 1 mM sodium pyruvate and 10 mM HEPES) at a density of 3x10<sup>6</sup> cells/ml or in a binding buffer (PBS

containing 0.1% bovine serum albumin and 0.02% NaN<sub>3</sub>, at a density of  $6 \times 10^5$  cells/ml.

IL-8 receptor binding assay:

Multiscreen filter plates (96-well, Millipore, MA) were pretreated with a PBS binding buffer containing 0.1% bovine serum albumin and 0.02% NaN<sub>3</sub> for 2 hours. A final volume of 150  $\mu$ l, containing  $4 \times 10^5$  neutrophils,  $0.25 \text{ nM}$  [<sup>125</sup>I]-human-IL-8 (Amersham, IM-249) and varying concentrations of antibodies made up in PBS binding buffer, was added to each well, and plates were incubated for 90 min at 4°C. Cells were washed 5 times with 200  $\mu$ l of ice-cold PBS, which was removed by aspiration. The filters were air-dried, 3.5 ml of scintillation fluid was added (Beckman Ready Safe) and filters were counted on a Beckman LS6000TC counter. The data obtained is presented as % specific bound [<sup>125</sup>I]-IL-8, which is calculated as the cpm in the presence of antibody divided by the cpm in the presence of PBS binding buffer only and multiplied by 100 (Figure 15). All six of the human anti-IL-8 monoclonals tested blocked IL-8 binding to human neutrophils.

Neutrophil CD11b (Mac-1) expression assay:

Human IL-8 at a final concentration of 10 nM was preincubated with varying concentrations of monoclonal antibodies at 4°C for 30 minutes and at 37°C for an additional 30 min. Neutrophils ( $4 \times 10^5$ /well) were exposed to IL-8 in the presence or absence of antibodies at 4°C for 90 min, and incubated with PE-conjugated mouse-anti-human-CD11b (Beckon Dickinson) for 45 min at 4°C. The cells were washed with ice-cold PBS containing 2% fetal calf serum. Fluorescence was measured on a Beckon Dickinson FACScan cell analyzer. A mouse monoclonal antibody against human CD11b obtained from R&D System, Inc. was used as a positive control while the purified myeloma human IgG2 (Calbiochem) was used as a negative control in the experiments. The expression levels of CD11b on neutrophils were measured and expressed as the mean fluorescence channel.

derived from the negative control antibody was subtracted from those of experimental samples.

5 mean fluorescence in the presence of IL-8 - the presence of antibodies only  
 % inhibition =  $\frac{\text{mean fluorescence in the presence of IL-8} - \text{mean fluorescence in the presence of human IgG2}}{\text{mean fluorescence in the presence of IL-8} - \text{mean fluorescence in the presence of human IgG2}} \times 100$

10

As shown in Table 5, five of the six antibodies blocked upregulation of CD11b to some degree, with three of the five giving complete blocking.

15

TABLE 5: Inhibition of CD11b expression on human neutrophils by monoclonal antibodies against IL-8.

Antibody	Concentration (nM)	Inhibition of CD11b expression (%)
R&D anti-IL8	333	100
18K1.1	6	100
18K2.1	10	60
18K2.2	32	100
18K4.2	3	10
18K4.3	8	100
18K4.5	5	0
Human IgG2	33	0

30 Background of CD11b expression is 670 (mean fluorescence) while CD11b expression in the presence of 10 nM of human IL-8 is 771.



Sequence analysis of immunoglobulin transcripts derived from anti-H1-8 hybridomas.

All sequences were derived by direct sequencing of PCR fragments generated from RT-PCR reactions of RNA prepared from hybridomas D1.1, K4.2 and K4.3, using human V<sub>H</sub> and human V<sub>L</sub> family specific primers (Marks et al. 1991; Euro J. Immunol 21:1985-991) and a primer specific for either the human gamma 2 constant region (MG-40d; 5'-GCTGAGGAGTGAAGTCTGAGGACTGT-3') or human kappa constant region (HKP2; Green et al 1994; Nature Genetics 7: 13-21).

In Figure 16 A-H, both strands of the four clones were sequenced and analyzed to generate the complete sequence. All sequences were analyzed by alignments to the "V BASE sequence directory", Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, UK. The variable and joining regions are indicated by brackets [ ]. Nucleotides containing an "N" indicate uncertainty in the generated sequence.

Based on sequence alignments with sequences found in the V-base database the heavy chain transcript from hybridoma D1.1 has a human V<sub>H</sub>-41(DP-63) variable region (7 point mutations were observed compared to the germline sequence), a human J<sub>H</sub>-3 joining region and a human gamma 2 constant region. See Figure 15A. The kappa light chain transcript from hybridoma D1.1 is comprised of a human kappa variable region with homology to V<sub>L</sub> 08/018 (DPK1) (16 point mutations were observed when compared to the germline sequence) a human J<sub>L</sub>-3 joining region, and a human kappa constant region. See Figure 15B. Based on sequence alignments with sequences found in the V-base database the heavy chain transcript from

hybridoma K2.2 has a human V<sub>H</sub>-30 variable region (3 point mutations were observed compared to the germline sequence), a human J<sub>H</sub>-3 joining region, a human kappa constant region. See Figure 16C. The kappa light chain transcript from hybridoma K2.2 is comprised of a human kappa variable region with homology to V<sub>L</sub> 14 (B3; DPK24) (9 point mutations were observed

when compared to the germline sequence), a human J<sub>H</sub>3 joining region, and a human kappa constant region. See Figure 16D. Based on sequence alignments with sequences found in the V-base database the heavy chain transcript from hybridoma K4.2 has a human V<sub>H</sub>4-34 variable region (8 point mutations were observed compared to the germline sequence), a human J<sub>H</sub>4 joining region, and a human kappa constant region. See Figure 16E. The kappa light chain transcript from hybridoma K4.2 is comprised of a human kappa variable region with homology to V<sub>H</sub>08/018 (DPK1) (6 point mutations were observed when compared to the germline sequence), a human J<sub>H</sub>4 joining region, and a human kappa constant region. See Figure 16F. Based on sequence alignments with sequences found in the V-base database the heavy chain transcript from hybridoma K4.3 has a human V<sub>H</sub>5-51 (DP-73) variable region, a human M5-a/M5-b D segment, a human J<sub>H</sub>4 joining region and a human gamma 2 constant region. See Figure 16G. The kappa light chain transcript from hybridoma K4.3 is comprised of a human kappa variable region with homology to V<sub>H</sub>02/012 (DPK9) (9 point mutations were observed when compared to the germline sequence), a human J<sub>H</sub>4 joining region, and a human kappa constant region. See Figure 16H. All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Biological Deposits

YHIC contained in S. cerevisiae was deposited with

the American Type Culture Collection ("ATCC"), 12301 Parklawn Drive, Rockville MD 20852, USA, on April 26, 1996, and given

ATCC accession no. \_\_\_\_\_. The deposit of this YAC is for exemplary purposes only, and should not be taken as an

admission by the Applicant that such deposit is necessary for enablement of the claimed subject matter.

In respect of all designated states in which such

action is possible and to the extent that it is legally

permissible under the law of the designated state, it is

requested that a sample of the deposited micro-organisms be

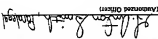
made available only by the issue thereof to an independent

expert, in accordance with the relevant patent legislation,

e.g., EPC rule 28(4), United Kingdom Patent Rules 1982 rule

17(3), Australian Regulation 3.25(3) and generally similar

provisions mutatis mutandis for any other designated state.

<p align="center"><b>MICROORGANISMS</b></p> <p align="center">Special Sheet in connection with the microorganism referred to on page 41, lines 1-20 of the description</p>	
<p><b>A. IDENTIFICATION OF DEPOSIT</b></p> <p>Further deposits are identified on an additional sheet</p>	
<p>Name of depositary institution</p> <p align="center">American Type Culture Collection</p>	
<p>Address of depositary institution (including postal code and country)</p> <p align="center">12301 Parklawn Drive Rockville, MD 20852 US</p>	
<p>Date of deposit: Apr 25, 1995 Accession Number</p>	
<p><b>B. ADDITIONAL INDICATIONS</b> - (Leave blank if not applicable). This information is inserted on a separate attached sheet</p>	
<p><b>C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> - (If no indication on an attached sheet)</p>	
<p><b>D. SEPARATE FURNISHING OF INDICATIONS</b> - (Leave blank if not applicable)</p>	
<p>This section below will be submitted to the International Bureau later - (Specify the general nature of the indication e.g., "Accession Number of Deposit")</p>	
<p><b>E. <u>is</u> This sheet was received with the International application when filed (to be checked by the receiving Office)</b></p> <p align="center">         (Authorized Officer)     </p> <p><input type="checkbox"/> The date of receipt (from the applicant) by the International Bureau</p> <p align="right">was</p> <p align="right">(Authorized Officer)</p>	
<p align="right">Form PCT/MC/154 (January 1981)</p>	

International Application No: PCT/

## CLAIMS

1. A method to produce a human immunoglobulin or an analog thereof, specific for a desired antigen, which method comprises:
  - administering said antigen or an immunogenic portion thereof to a nonhuman animal under conditions to stimulate an immune response, whereby said animal produces B cells that secrete immunoglobulin specific for said antigen; wherein said nonhuman animal is characterized by being substantially incapable of producing endogenous heavy and light immunoglobulin chains, but capable of producing human immunoglobulin; and
  - recovering said immunoglobulin or analog.
2. The method of claim 1 wherein said recovering step comprises recovering polyclonal immunoglobulin or analog from said animal.
3. The method of claim 1 wherein said recovering step comprises immortalizing B cells from said animal immunized with said antigen, screening the resulting immortalized cells for the secretion of said immunoglobulin specific for said antigen, and
- a) recovering immunoglobulin secreted by said immortalized B cells, or
- b) recovering the genes encoding at least the immunoglobulin from the immortalized B cells, and optionally modifying said genes;
- expressing said genes or modified forms thereof to produce immunoglobulin or analog; and
- recovering said immunoglobulin or analog.
4. The method of claim 1 wherein said recovering step comprises:
  - recovering genes encoding the immunoglobulins from the primary B cells of the animal;

- generating a library of said genes expressing the immunoglobulins;  
 screening the library for an immunoglobulin with the desired affinity for the antigen;  
 recovering the genes encoding the immunoglobulin; expressing said recovered genes to produce an immunoglobulin or analog recovering said immunoglobulin or analog.
5. A recombinant DNA molecule comprising a nucleotide sequence encoding the immunoglobulin or analog produced by the method of claim 1.
6. The DNA molecule of claim 5 wherein said encoding-nucleotide sequence is operably linked to control sequences capable of effecting its expression.
7. A cell or cell line modified to contain the DNA molecule of claim 6.
8. A method to produce a fully human immunoglobulin or an analog thereof which method comprises culturing the cells of claim 7 under conditions whereby said encoding nucleotide sequence is expressed to produce said immunoglobulin or analog; and recovering said immunoglobulin or analog.
9. A DNA molecule comprising a nucleotide sequence corresponding to the gene or modified gene prepared by the method of claim 3.
10. The DNA molecule of claim 9 wherein said encoding nucleotide sequence is operably linked to control sequences capable of effecting its expression.
11. A cell or cell line modified to contain the DNA molecule of claim 9.

12. A method to produce a fully human immunoglobulin or an analog thereof which method comprises culturing the cells of claim 11 under conditions whereby said encoding nucleotide sequence is expressed to produce said immunoglobulin or analog; and recovering said immunoglobulin or analog.
13. A DNA molecule which comprises a nucleotide sequence encoding a human immunoglobulin with desired affinity prepared according to the method of claim 4.
14. The DNA molecule of claim 13 wherein said encoding nucleotide sequence is operably linked to control sequences capable of effecting its expression.
15. A cell or cell line modified to contain the DNA molecule of claim 13.
16. A method to produce a fully human immunoglobulin or an analog thereof which method comprises culturing the cells of claim 15 under conditions whereby said encoding nucleotide sequence is expressed to produce said immunoglobulin or analog; and recovering said immunoglobulin or analog.
17. An immortalized B cell which secretes a fully human immunoglobulin to a desired antigen prepared according to claim 3.
18. A method to produce an immunoglobulin or analog which comprises culturing the cells of claim 17 and recovering said immunoglobulin or analog.
19. A fully human immunoglobulin or analog produced by the method of claim 1.

20. The immunoglobulin or analog of claim 19 wherein the desired antigen is selected from the group consisting of

the leukocyte markers, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD11a,b,c, CD13, CD14, CD18, CD19, CD20, CD22, CD23, CD27 and its ligand, CD28 and its ligands B7.1, B7.2, B7.3, CD29 and its ligand, CD30 and its ligand, CD40 and its ligand gp39, CD44 and isoforms, CD52 (campath antigen), CD56, CD58, CD59, CD72, CD134-4, LFA-1 and TCR;

the Lewis x antigens, Slex, Sley, Slea, and Slob;

the integrins, VIA-1, VIA-2, VIA-3, VIA-4, VIA-5, VIA-6,  $\alpha V\beta 3$ , and LFA-1, Mac-1, and p150<sup>95</sup>,  $\alpha\beta$ , gp130,  $\alpha\beta$ ,  $\alpha\beta$ ,  $\alpha\beta$ ,  $\alpha\beta$ , and  $\alpha\beta$ ;

the selectins, L-selectin, P-selectin, and E-selectin and their counterreceptors VCAM-1, ICAM-1, and LFA-3;

the interleukins, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, and IL-15;

the interleukin receptor is selected from the group consisting of IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-8R, IL-9R, IL-10R, IL-11R, IL-12R, IL-13R, IL-14R, and IL-15R;

the chemokine is selected from the group consisting of RANTES, MIP1a, MCP1, NAP-2, GRO $\alpha$ , GRO $\beta$ , and IL-8;

the growth factor is selected from the group consisting of TNF $\alpha$ , TNF $\beta$ , TGF $\beta$ , TGF $\beta$ , VEGF/VPE, PDGF, EGF family, TGF $\beta$ , PDGF family, endostatin, fibronectin (F $\beta$ ), human laminin, and gastrin releasing peptide (GRP);

the growth factor receptor is selected from the group consisting of TNF $\alpha$ phar, TNF $\beta$ phar, NGFphar, VEGFR/VPE, PDGF, EGF, PDGFR, PDGFR family, EPO-R, GCSF-R and other hematopoietic receptors;

the interferon receptor is selected from the group consisting of IFN $\alpha$ R, IFN $\beta$ R, and IFN $\gamma$ R;

the Ig and its receptor is selected from the group consisting of IgE, IgG1, and IgG2;



- the tumor antigen is selected from the group consisting of her2-neu, mucin, CEA and endostatin; the allergen is selected from the group consisting of house dust mite antigen, Iol PI (grass) antigens, and urushiol;
- 5 the viral protein is selected from the group consisting of CMV glycoproteins B, H, and gCIII, HIV-1 envelope glycoproteins, RSV envelope glycoproteins, HSV envelope glycoproteins, HPV envelope glycoproteins, Hepatitis family surface antigens;
- 10 the toxin is selected from the group consisting of pseudomonas endotoxin and osteopontin/irapontin, snake venom, spider venom, and bee venom conotoxin;
- 15 the blood factor is selected from the group consisting of complement C3b, complement C4a, complement C4b-9, Rh factor, fibrinogen, fibrin, and myelin associated growth inhibitor; and
- the enzyme is selected from the group consisting of cholesterol ester transfer protein, membrane bound matrix metalloproteinases, and glutamic acid decarboxylase (GAD).
- 20 21. The immunoglobulin or analog of claim 14 wherein said desired antigen is selected from the group consisting of human IL-6, human IL-8, human TNF $\alpha$ , human CD4, human L-selectin, human gp39, human IgG, human  $\alpha$ V $\beta$ 3, human fibronectin (FNC), human laminin, human PTHrP, and tetanus toxin C(IVC).
22. A recombinant DNA molecule comprising a nucleotide sequence that encodes the immunoglobulin or analog of claim 19-21.
23. The DNA molecule of claim 22 wherein said encoding nucleotide sequence is preferably linked to control sequences capable of effecting its expression.
24. A cell or cell line modified to contain the DNA molecule of claim 23.

25. A method for the production of an immunoglobulin for a desired antigen which method comprises culturing the cell in cell line of claim 24 under conditions where the nucleotide sequence expressed or produced is different from the sequence expressed or produced in the cell prior to the introduction of the foreign DNA into the cell.

26. An human antibody or analog thereof which is specifically immunoreactive with an antigen selected from the group consisting of: transfection state markers; leukocyte markers; histocompatibility antigens; adhesion molecules; interleukins; interleukin receptors; chemokines; growth factors; growth factor receptors; interferon receptors; IgG and their receptors; tumor antigens; allergens; viral proteins; toxins; blood factors; enzymes; and the miscellaneous antigens ganglioside GD3, ganglioside GB2, LMW1, LMW2, eosinophil major basic protein, eosinophil cationic protein, pANCA, Yamadori protein, Type IV collagen, glycosylated lipids,  $\gamma$ -interferon,  $\gamma$ 7, P-glycoprotein, Fas (APO-1) and oxidized-IDL.

27. The antibody or analog of claim 26 wherein the leukocyte marker is selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD11a,b,c, CD13, CD14, CD18, CD19, CD20, CD22, CD23, CD27 and its ligand, CD28 and its ligands B7.1, B7.2, B7.3, CD29 and its ligand, CD30 and its ligand, CD40 and its ligand gp39, CD44, CD45 and isoforms, CDw52 (Campath antigen), CD56, CD58, CD69, CD72, CD73, CD74, CD75, CD76, CD77, CD78, CD79, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103, CD104, CD105, CD106, CD107, CD108, CD109, CD110, CD111, CD112, CD113, CD114, CD115, CD116, CD117, CD118, CD119, CD120, CD121, CD122, CD123, CD124, CD125, CD126, CD127, CD128, CD129, CD130, CD131, CD132, CD133, CD134, CD135, CD136, CD137, CD138, CD139, CD140, CD141, CD142, CD143, CD144, CD145, CD146, CD147, CD148, CD149, CD150, CD151, CD152, CD153, CD154, CD155, CD156, CD157, CD158, CD159, CD160, CD161, CD162, 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CD592, CD593, CD594, CD595, CD596, CD597, CD598, CD599, CD600, CD601, CD602, CD603, CD604, CD605, CD606, CD607, CD608, CD609, CD610, CD611, CD612, CD613, CD614, CD615, CD616, CD617, CD618, CD619, CD620, CD621, CD622, CD623, CD624, CD625, CD626, CD627, CD628, CD629, CD630, CD631, CD632, CD633, CD634, CD635, CD636, CD637, CD638, CD639, CD640, CD641, CD642, CD643, CD644, CD645, CD646, CD647, CD648, CD649, CD650, CD651, CD652, CD653, CD654, CD655, CD656, CD657, CD658, CD659, CD660, CD661, CD662, CD663, CD664, CD665, CD666, CD667, CD668, CD669, CD670, CD671, CD672, CD673, CD674, CD675, CD676, CD677, CD678, CD679, CD680, CD681, CD682, CD683, CD684, CD685, CD686, CD687, CD688, CD689, CD690, CD691, CD692, CD693, CD694, CD695, CD696, CD697, CD698, CD699, CD700, CD701, CD702, CD703, CD704, CD705, CD706, CD707, CD708, CD709, CD710, CD711, CD712, CD713, CD714, CD715, CD716, CD717, CD718, CD719, CD720, CD721, CD722, CD723, CD724, CD725, CD726, CD727, CD728, CD729, CD730, CD731, CD732, CD733, CD734, 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the histocompatibility antigen is selected from the group consisting of MHC class I or II, the Lewis y antigens, Sley, Sley, and Sley;

the adhesion molecule is selected from the group

the adhesion molecule is selected from the group consisting of VLA-1, VLA-2, VLA-3, VLA-4, VLA-5, VLA-6,  $\alpha$ 4 $\beta$ 3,  $\alpha$ 4 $\beta$ 1, and LFA-1, Mac-1, p150<sup>95</sup>,  $\alpha$ 6 $\beta$ 1, gp130,  $\alpha$ 5 $\beta$ 1,  $\alpha$ 6 $\beta$ 1,  $\alpha$ 6 $\beta$ 2,  $\alpha$ 6 $\beta$ 3,  $\alpha$ 6 $\beta$ 4,  $\alpha$ 6 $\beta$ 5,  $\alpha$ 6 $\beta$ 6,  $\alpha$ 6 $\beta$ 7,  $\alpha$ 6 $\beta$ 8,  $\alpha$ 6 $\beta$ 9,  $\alpha$ 6 $\beta$ 10,  $\alpha$ 6 $\beta$ 11,  $\alpha$ 6 $\beta$ 12,  $\alpha$ 6 $\beta$ 13,  $\alpha$ 6 $\beta$ 14,  $\alpha$ 6 $\beta$ 15,  $\alpha$ 6 $\beta$ 16,  $\alpha$ 6 $\beta$ 17,  $\alpha$ 6 $\beta$ 18,  $\alpha$ 6 $\beta$ 19,  $\alpha$ 6 $\beta$ 20,  $\alpha$ 6 $\beta$ 21,  $\alpha$ 6 $\beta$ 22,  $\alpha$ 6 $\beta$ 23,  $\alpha$ 6 $\beta$ 24,  $\alpha$ 6 $\beta$ 25,  $\alpha$ 6 $\beta$ 26,  $\alpha$ 6 $\beta$ 27,  $\alpha$ 6 $\beta$ 28,  $\alpha$ 6 $\beta$ 29,  $\alpha$ 6 $\beta$ 30,  $\alpha$ 6 $\beta$ 31,  $\alpha$ 6 $\beta$ 32,  $\alpha$ 6 $\beta$ 33,  $\alpha$ 6 $\beta$ 34,  $\alpha$ 6 $\beta$ 35,  $\alpha$ 6 $\beta$ 36,  $\alpha$ 6 $\beta$ 37,  $\alpha$ 6 $\beta$ 38,  $\alpha$ 6 $\beta$ 39,  $\alpha$ 6 $\beta$ 40,  $\alpha$ 6 $\beta$ 41,  $\alpha$ 6 $\beta$ 42,  $\alpha$ 6 $\beta$ 43,  $\alpha$ 6 $\beta$ 44,  $\alpha$ 6 $\beta$ 45,  $\alpha$ 6 $\beta$ 46,  $\alpha$ 6 $\beta$ 47,  $\alpha$ 6 $\beta$ 48,  $\alpha$ 6 $\beta$ 49,  $\alpha$ 6 $\beta$ 50,  $\alpha$ 6 $\beta$ 51,  $\alpha$ 6 $\beta$ 52,  $\alpha$ 6 $\beta$ 53,  $\alpha$ 6 $\beta$ 54,  $\alpha$ 6 $\beta$ 55,  $\alpha$ 6 $\beta$ 56,  $\alpha$ 6 $\beta$ 57,  $\alpha$ 6 $\beta$ 58,  $\alpha$ 6 $\beta$ 59,  $\alpha$ 6 $\beta$ 60,  $\alpha$ 6 $\beta$ 61,  $\alpha$ 6 $\beta$ 62,  $\alpha$ 6 $\beta$ 63,  $\alpha$ 6 $\beta$ 64,  $\alpha$ 6 $\beta$ 65,  $\alpha$ 6 $\beta$ 66,  $\alpha$ 6 $\beta$ 67,  $\alpha$ 6 $\beta$ 68,  $\alpha$ 6 $\beta$ 69,  $\alpha$ 6 $\beta$ 70,  $\alpha$ 6 $\beta$ 71,  $\alpha$ 6 $\beta$ 72,  $\alpha$ 6 $\beta$ 73,  $\alpha$ 6 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$\beta$ 195,  $\alpha$ 6 $\beta$ 196,  $\alpha$ 6 $\beta$ 197,  $\alpha$ 6 $\beta$ 198,  $\alpha$ 6 $\beta$ 199,  $\alpha$ 6 $\beta$ 200,  $\alpha$ 6 $\beta$ 201,  $\alpha$ 6 $\beta$ 202,  $\alpha$ 6 $\beta$ 203,  $\alpha$ 6 $\beta$ 204,  $\alpha$ 6 $\beta$ 205,  $\alpha$ 6 $\beta$ 206,  $\alpha$ 6 $\beta$ 207,  $\alpha$ 6 $\beta$ 208,  $\alpha$ 6 $\beta$ 209,  $\alpha$ 6 $\beta$ 210,  $\alpha$ 6 $\beta$ 211,  $\alpha$ 6 $\beta$ 212,  $\alpha$ 6 $\beta$ 213,  $\alpha$ 6 $\beta$ 214,



9, RN factor, fibrinogen, fibrin, and myelin associated growth inhibitor; and

The enzyme is selected from the group consisting of cholesterol ester transfer protein, membrane bound matrix metalloproteases, and glutamic acid decarboxylase (GAD)

28. The antibody or analog of claim 26 wherein the desired antigen is selected from the group consisting of human IL-6, human TNF $\alpha$ , human CD4, human L-selectin, human gp39, human IgE and tetanus toxin C (TTC).

29. The antibody or analog of claim 19 wherein the desired antigen is human IL-6.

30. The antibody or analog of claim 19 wherein the described antigen is human IL-8.

31. The antibody or analog of claim 19 wherein the desired antigen is human TNF $\alpha$ .

32. The antibody or analog of claim 19 wherein the desired antigen is human CD4.

33. The antibody or analog of claim 19 wherein the desired antigen is human L-selectin.

34. The antibody or analog of claim 19 wherein the desired antigen is human gp39.

35. The antibody or analog of claim 19 wherein the desired antigen is tetanus toxin C (TTC).

36. The antibody or analog of claim 19 wherein the desired antigen is human IgE.

37. The antibody or analog of claim 19 wherein the desired antigen is human  $\alpha\beta 3$ .

38. The antibody or analog of claim 19 wherein the desired antigen is human fibronectin.
39. The antibody or analog of claim 19 wherein the desired antigen is human PTHrp.
40. The antibody or analog of claim 26 which is an agonist or is a catalyst.
41. A recombinant DNA molecule encoding the antibody of any of claim 26-40.
42. A recombinant DNA molecule which comprises an expression system for the antibody or analog of any claims 26-40 which expression system comprises a nucleotide sequence encoding said antibody or analog operably linked to control sequences capable of effecting its expression.
43. A recombinant host cell which is modified to contain the DNA molecule of claim 42.
44. A method to produce an antibody or analog which method comprises culturing cells of claim 43 under conditions wherein said coding sequence is expressed; and recovery the antibody of analog produced.

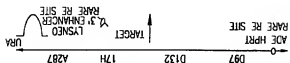
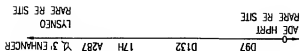


FIG. 1

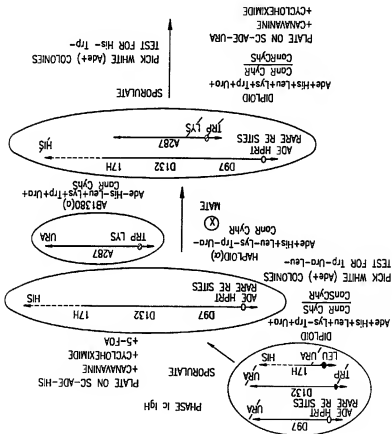




FIG. 2

FIG.4

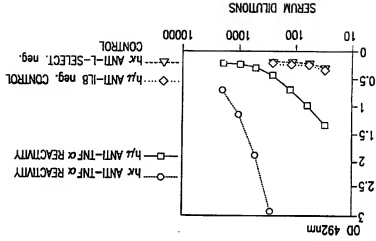
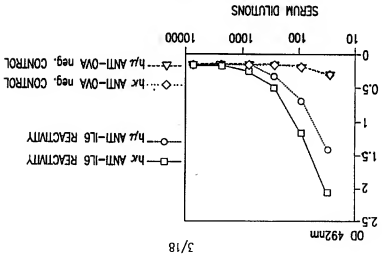
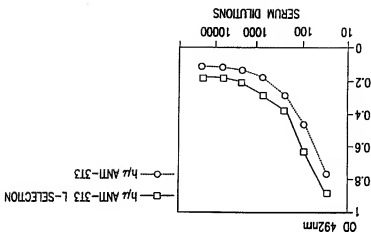
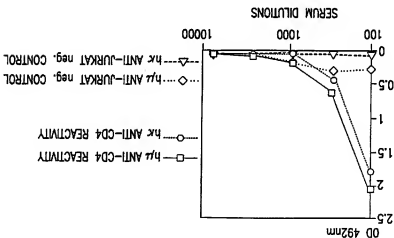


FIG.3





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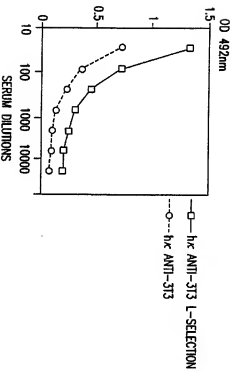


FIG. 7

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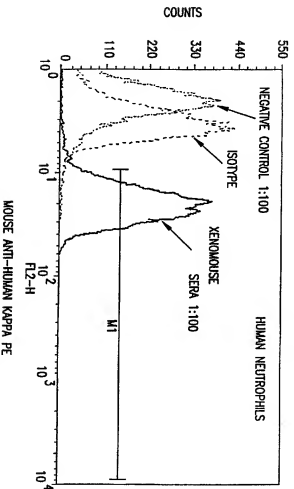


FIG. 8

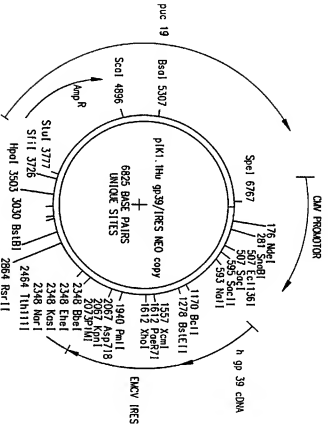


FIG.9

FIG. 11

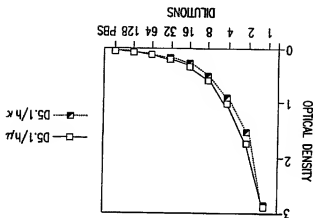


FIG. 10

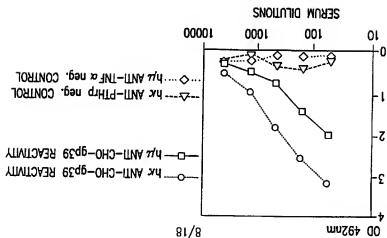
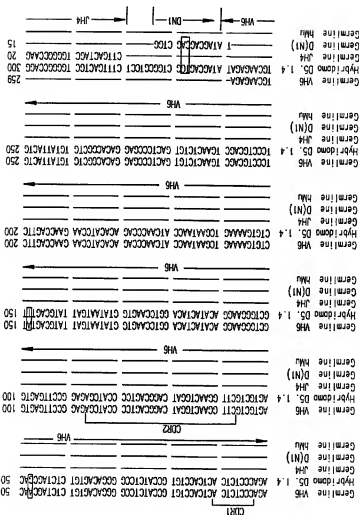


FIG. 12A



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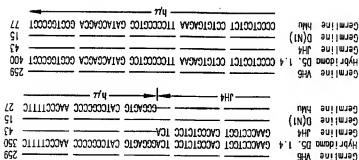


FIG. 12B

FIG. 13A





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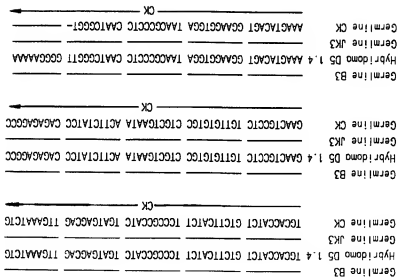


FIG. 13B

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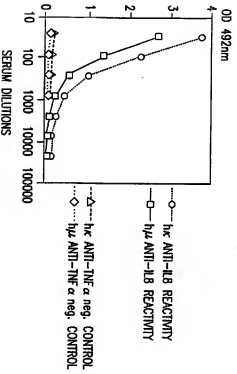
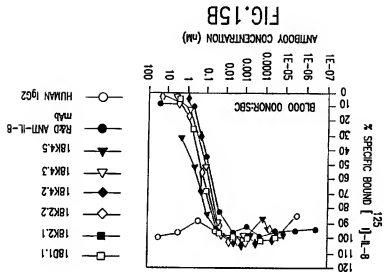
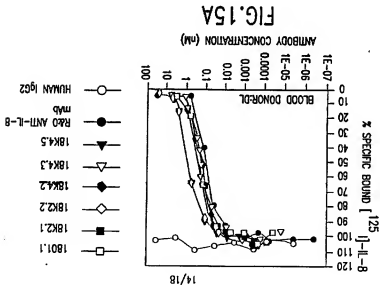


FIG.14



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[CCTGTCCCTCACCCTGCGCTGTCTATATGATGGGTGCTTCAAGTGGTTACTACTGGAAGCTGGATTCGCGC  
 AGCCCCCAAGGGAGTGGAGTGGATTGGGAAATCAATCAAGTGGAAAGCAACCAATTACAA  
 CCGGTCCCTCAAGAGTGGATCATATATCAATAGACACGTCCAAAGACCCAGTTCCTCCCTGGAAGT  
 TGAECTCTGTGACCCGCGCGGACACGCGCTGTGTATTACTGTGTGAGAGAG][GACTCCCC][ATGCT  
 TTGATATCTGGGGCCCAAGGAGCAATGGTCAACGCTCTCTTAG][CCTCCACCAAGGGCCCATCG  
 TCTTCCCCCTGGCGCCCTGCTCCAGGAGCACACTCCGAGAGGACAGC(GC)GCCCCTGGGCTGCTG  
 GTCAAGGACTACTTCC

FIG. 16A

[CAGTCTCCATCTCTCCCTGTGTCATCTGTAGGCGACAGAGTCACCATCTGTCAGGGGAGTGC  
 AGACATTTAGTTAAGTTTTTAAGTTGGTTTCAACAGAAACCAAGGAAAGCCCCCTAAACTCCTGATC  
 TACGGTACATCTCTATTTGGAAACCGGGGCTCCCATCAAGTTTCAGTGGAAAGTGGATCTGGGACAGAG  
 TTTTACTCTCACCATCAAGCAGCTGCAAGCTGTGCAACATATTTCTGTAAACAGNATG  
 ATGATCTGCC][ATACACTTTTGGGCCCTGGGACCAAAAGTGGATATCAAAAC]GAACTGTGCTGCAC  
 CATCTGTCTTCATCTTCCCGCCATCTGATGATGAGCAGTTGAAATCTGGAACTGCTGTGTGTGTGCTG  
 TGGTGAATAACTTCTATCTCCAGAGGCCCAAAAGTACAGTGGAAAGTGGATTAACGCC

FIG. 16B

SUBSTITUTE SHEET (RULE 26)

FIG. 16C

[illegible]

FIG. 16D



AGTCTCTGAAAGATCTCCCTGTAAGGGCTCTCGATACAGCTTACCAAGCTACTGATCGGCTGGGTG

CGCCAGATGCCCGGGGMMAGTGGATGGGATCATCTATCTCTGCTGACTCTGATACCA

GATACAGCCCGTCTTCCAGGCCAGGTCAACATTCAGGCGACATGTCATCAGCACCGCTA

CC TGCAGTGGAGCAGCTTGAGGBCCTCGGACACCGCCATGATTACTGTGCGAGACA][GGACGG

[TG][ACTCCCTTGGACTACTGAGGGGCCAGGGAAACCTCGATCACCCTCTCTCAg]CCTCCACCAAGGG

CCCATCGAGTCTTCCTCCCTGGGACCTGGCCTCCAGGAGCAACTCCGAGAGCACAGC(GC)GGCCCTG

gagctggctggatccaggacacttcccccggacc006tga0095tgt05tggaaactcagggccgctct

GAC CAG CGG CGT GCAC ACCTT CCACT GCA

FIG. 16H

TGCTTCATCTATTGGAGACAGAGTTCACCATCATTGCCGGGCAAGTCAGAGCATTAGCAACTA

TTTAAATTGGTATTCAGCAGAAACAGGGCAAAAGCCCTTAAAGTTCTGATCTATGGTGCATCCAGT

TTGGAAAGTGGGGTCCCATCAGGTTTCAGTGGCAGTGGATCTGGGACACAGATTTCTCCTCAGCAT

CAAGAGCC TGCACCTGNGA TTTGCACTTACTACTGTCACAGAGTTACAGTAACCC TTTCTC

ACTTTGGGAGCCAAATGTCACCAAC[GACTGTGGCTGCACCATTGTCTTCACT

TCCGCGCACTGGAGCAGTTGAAATCTGGACTGCTTGTGTCCTGCTGAATACTCT

ATCCAGAGAGGCTAAGTACA

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/05928

<p><b>A. CLASSIFICATION OF SUBJECT MATTER</b>          IPC69: A61K 39/00; C07K 16/18; C12N 5/16, 15/13          US CL: A61K184.1; 514/12; 536/23.53; 530/287.1          Minimum documentation searched (classification symbols)          U.S. : 424/184.1; 514/12; 536/23.53; 530/287.1</p>		<p><b>B. FIELD(S) SEARCHED</b>          According to International Patent Classification (IPC) or to both national classification and IPC</p>	
<p><b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b></p>			
<p>Electronic data have been consulted during the international search (name of data base used, where practicable, search terms used)</p>			
<p>Documentation searched other than information documentation to the extent that such documents are included in the fields searched</p>			
<p>APS, DIALOG</p>			
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
X	EP 463151 A, (JAKOBOVITZ ET AL) 01 February 1991, See abstract.	1-4, 19	1-44
Y			
Y, X	Expert Opinion on Investigational Drugs, Volume 3, Number 3, issued March 1994, Emery et al., "Humanised monoclonal antibodies for therapeutic applications", pages 241-251, especially pages 243-245.	20-44	
<p><input type="checkbox"/> Further documents are based on the documentation of Box Y</p>			
<p><input type="checkbox"/> See patent family links</p>			
<p>* Category of documents is indicated by the following:</p>			
X	Documents of the international patent classification (IPC) class A61K 39/00, C07K 16/18, C12N 5/16, 15/13, and C12N 5/10, 15/13, 15/14, 15/15, 15/16, 15/17, 15/18, 15/19, 15/20, 15/21, 15/22, 15/23, 15/24, 15/25, 15/26, 15/27, 15/28, 15/29, 15/30, 15/31, 15/32, 15/33, 15/34, 15/35, 15/36, 15/37, 15/38, 15/39, 15/40, 15/41, 15/42, 15/43, 15/44, 15/45, 15/46, 15/47, 15/48, 15/49, 15/50, 15/51, 15/52, 15/53, 15/54, 15/55, 15/56, 15/57, 15/58, 15/59, 15/60, 15/61, 15/62, 15/63, 15/64, 15/65, 15/66, 15/67, 15/68, 15/69, 15/70, 15/71, 15/72, 15/73, 15/74, 15/75, 15/76, 15/77, 15/78, 15/79, 15/80, 15/81, 15/82, 15/83, 15/84, 15/85, 15/86, 15/87, 15/88, 15/89, 15/90, 15/91, 15/92, 15/93, 15/94, 15/95, 15/96, 15/97, 15/98, 15/99, 15/100, 15/101, 15/102, 15/103, 15/104, 15/105, 15/106, 15/107, 15/108, 15/109, 15/110, 15/111, 15/112, 15/113, 15/114, 15/115, 15/116, 15/117, 15/118, 15/119, 15/120, 15/121, 15/122, 15/123, 15/124, 15/125, 15/126, 15/127, 15/128, 15/129, 15/130, 15/131, 15/132, 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